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Sequential Nitric Oxide Measurements During the Emergency Department Treatment of Acute Vasoocclusive Sickle Cell Crisis

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This prospective study was designed to examine the relationship between serial serum nitric oxide (NO) levels and pain during the emergency department (ED) treatment of acute vasoocclusive sickle cell crisis (SCC). 102 patient visits, age ≥18 years of age, presenting to the ED with uncomplicated, typical SCC pain had serum NO levels obtained at 2-hr intervals during treatment of pain and were measured using an NO-specific chemiluminesence technique. Pain was measured prior to each NO measurement using a 10 cm visual analog scale (VAS), and subjects were divided into a persistent pain group and an improved pain group. Patients with persistent pain had significantly low initial NO levels (11.51 μM ± 2.8, $P < 0.05$) while those with pain improvement had higher initial NO levels (18.1 μM ± 3.08, $P < 0.05$). There was no significant correlation between changes in NO and changes in pain scores. These results suggest that the initial NO level may serve as a marker for the severity of tissue ischemia. Sequential NO levels do not appear useful in predicting the course of SCC. Am. J. Hematol. 64:15–19, 2000.

Key words: sickle cell anemia; nitric oxide; pain

INTRODUCTION

Acute vasoocclusive sickle cell crisis (SCC) is the most common disease presentation of adult sickle cell anemia in the emergency department (ED) [1]. SCC is characterized by acute tissue ischemia secondary to local microvascular occlusion and hypoxia that results from sickled red blood cells [2]. Current ED treatment typically involves parenteral analgesic administration until pain resolves.

One important physiologic response to tissue ischemia is vasodilation. Nitric oxide (NO) is an endogenously-produced vasodilator, produced largely by the vascular endothelium, that plays a major regulatory role in both physiologic and pathologic vascular states [3,4]. Physiologically, constitutive NO is regularly produced by the vascular endothelium where it acts as a potent endogenous vasodilator, a neutrophil accumulation inhibitor, and a platelet aggregation inhibitor [5,6]. In certain pathologic states, endothelial NO production can be diminished, leading to vasospasm and thrombosis, or it may be increased, as is found in sepsis, leading to vasodilation and hypotension [7,8,9]. Only two studies to date have measured NO levels in SCC. Rees et al [10] determined that mean plasma concentrations of the metabolites of NO were elevated in admitted SCC patients when compared to healthy, non-sickle cell volunteers. Our prior study suggested an inverse relationship between NO level and severity of SCC pain in ED patients [11]. Sickle cell crisis patients with high initial pain scores in the emergency department had lower NO levels, while those with lower initial pain scores had higher NO levels. Given the recent use of NO therapy for acute crisis, the measurement of NO may have diagnostic and therapeutic benefit [12].

We hypothesize that elevations in NO may represent a physiologic, compensatory response in SCC while lower

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NO levels may represent a subset of SCC patients with impaired vasodilation with resultant prolonged or worsened tissue ischemia. Our objective was to 1) determine the relationship of the initial NO level with pain severity in ED patients presenting with uncomplicated, acute vasoocclusive sickle cell crisis, and 2) determine if NO levels change during the course of ED analgesic therapy.

MATERIALS AND METHODS

Thomas Jefferson University Hospital has an annual census of 52,000 ED patient visits per year. Approximately 2,000 patient visits involve sickle cell anemia patients, with 98% representing acute, uncomplicated SCC. This study was approved by the Institutional Review Board of Thomas Jefferson University.

Adult SCC patients, age ≥ 18 years of age with a chief complaint of typical SCC pain was studied. Typical crisis pain was defined as pain consistent in duration, severity, quality, and distribution with prior episodes of SCC. All patients had prior documentation of sickle cell SS disease (determined by hemoglobin electrophoresis on cellulose acetate, citrate agar, and isoelectric focusing in the Thomas Jefferson University's Sickle Cell center). Excluded were patients <18 years of age, atypical pain, refusal to enroll in the study, prior entry into the study within 7 days, or evidence of acute, co-existing illness. Acute, co-existing illness was defined as an infectious illness, by history, of <7 days duration along with any of the following abnormal vital signs upon presentation: fever >101 °F, tachycardia >120 beats/min, tachypnea >30 breaths/min, and/or systolic BP <100 mmHg. The exclusion criteria were independent and exclusive (i.e., the presence of any one of the criteria was cause for exclusion from the study). Demographic, historical, and physical examination data was recorded. Specific historical information important to the study included time of onset and location of SCC pain.

After informed consent was obtained, the severity of pain was assessed by use of a 100 millimeter continuous visual analog pain scale (VAS). The left-side, zero point represented "no pain" while the right-side, 100 mm point represented "the worst pain I've ever had." The pain score was measured from left to right in mm. After the initial pain score was obtained, a 20–22 gauge intravenous catheter was inserted into an extremity vein. 4 cc of blood was then drawn into a heparinized tube for NO analysis. The measurement of pain score followed by blood sampling occurred at two-hour intervals, prior to analgesic administration, for up to a total of three measurements.

A control group ("Sickle cell control") included SS patients in their usual steady state.

NO Measurement

Blood samples were immediately centrifuged at 10,000 RPM for 10 minutes. The serum was separated and stored at −20°C.

NO is a soluble gas with a half-life of 3–15 seconds, making in vivo measurement of NO levels extremely difficult and impractical. The use of nitrite and nitrate (the stable end products of NO) measurements to quantify NO levels is commonly used in both animals [13] and humans [14,15] and has been well-documented.

Nitrite and nitrate levels were measured using the vanadium III reduction method [16,17]. Briefly, 50 μl of plasma was injected into a water-jacketed, oxygen-free, purge vessel containing 5 ml of 0.1 M vanadium III chloride (Aldrich, Milwaukee, WI) in 2 N HCl (Sigma). Acidic vanadium III at 98° C quantitatively reduces both nitrite and nitrate to NO, which is then quantified by a chemiluminescence detector (SIEVERS 270B Nitric Oxide Analyzer, Boulder, CO) after reaction with ozone. Signals from the detector were collected and analyzed using a PC-based data recording and processing system (Duo-18, World Precision Instruments, Inc., Sarasota, FL). Standard curves were obtained using the area under the curve after each injection of 10 μl of 0, 12.5, 25, 50, 75, and 100 μM sodium nitrate. The calculations to determine the nitric oxide content of the plasma are done by the slope of the regression analysis using the linear formula $y = a + bx$.

Data Analysis

Subjects were divided into Group 1, persistent pain, defined as an overall VAS change of <13 mm, and Group 2, improved pain, defined as ≥13 mm change in VAS during ED treatment. This division was based on the minimally significant change in patient pain severity study done by Todd et al. [18].

Visual analog pain scores were tested with the Kolmogorov-Smirnov test for normality and the Levene Median test for equal variance prior to analysis. Mean NO levels were analyzed with repeated-measures analysis of variance (ANOVA). NO levels were correlated to VAS scores using Pearson's correlation coefficient (r). Statistical significance was set at $p < 0.05$.

RESULTS

102 patient visits, representing 50 individual patients, were examined. 18 patients were entered into the control group. Table I presents the initial NO level by pain group. Patients with minimal to no change in pain during their emergency department treatment (Group 1—persistent pain) had significantly low initial NO levels.

Table II demonstrates a significant negative correlation between initial NO level and initial pain score in patients

TABLE I. Initial Nitric Oxide Levels

	Control (n = 18)	Group 1 SCC (n = 48)	Group 2 SCC (n = 54)
Initial NO (μM)	22.63 ± 2.5	11.51 ± 2.8*	18.1 ± 3.08
Age (yr)	25.0 ± 5.1	31.41 ± 10.9	32.8 ± 12.6

*P < 0.05 vs control, ANOVA.

TABLE II. Initial NO Level and Pain Score

Patient	No. of visits	r
A	4	−0.972*
B	5	−0.933*
C	6	−0.951*
D	6	−0.734*

*P < 0.05.

who were entered into the study most frequently (≥4 visits). In the remaining 81 visits analyzed, no meaningful correlation was found (r = 0.43, $p > 0.05$).

Table III shows the relationship between duration of pain prior to ED visit and initial NO level. There was a trend towards higher NO levels with increasing time prior to ED presentation, but this did not achieve statistical significance ($p = 0.10$).

There was no significant correlation between change in pain and change in NO levels at either the two-hour intervals or during the overall treatment period (r = 0.24, $p > 0.05$). In addition, there was no significant relationship between NO levels and location of pain or hemoglobin level. There was trend towards lower NO levels in those admitted to the hospital (6.5 μM ± 3.4 vs. 12.2 μM ± 4.5, $p > 0.05$) but this did not achieve statistical significance.

TABLE III. Initial NO Level and Duration of Pain

Duration of pain prior to ED visit	Initial NO level
<1 day	7.14 ± 4.3
1–3 days	11.49 ± 5.7
>3 days	12.41 ± 2.8

DISCUSSION

Acute, painful, sickle cell vasoocclusive crisis represents the most common presentation of adult SS patients to the ED [19,20]. During conditions of low oxygen tension, sickled RBCs become rigid and adhere to the capillary endothelial cells [21], causing obstruction of capillary blood flow, local tissue hypoxia, and further deoxygenation and sickling [2], thereby leading to large areas of ischemia and infarction.

The normal physiologic vascular response to ischemia is vasodilation [22,23], a response which attempts to restore adequate oxygen to the ischemic area. Nitric oxide (NO) is a significant vasodilator that is produced constitutively by the vascular endothelium, resulting in a baseline vasodilator state [3] in mammals. During ischemia, NO has been shown to play a significant role in protection against injury in a variety of mechanisms. Most importantly, as a vascular effector, NO causes significant vasodilation, resulting in increased blood flow to the ischemic area, thereby increasing oxygen and nutrient supply. Other studies have shown that NO attenuates tissue ischemia via inhibition of neutrophil activation [7], adhesion [8], and accumulation as well as platelet adhesion and aggregation [9], thereby ensuring an adequate blood flow. It appears that NO plays a significant role in the pathophysiology of sickle cell anemia. Mosseri et al [24] measured the vasorelaxation response of rabbit aorta strips to erythrocytes from SS patients. They found significant inhibition of endothelium-dependent vasorelaxation and attributed this to inhibition of NO. Rees et al [10] measured plasma NO levels in patients admitted to the hospital with acute SCC. In their study, they found significantly high NO levels compared to healthy, non-SS controls. There was, however, no difference in NO levels between SCC and SS "steady-state" controls. They postulated that, in SCC, the occlusion of the microvasculature may lead to an increase in NO production in an attempt to avoid tissue infarction. Our prior study examined a one-time, initial NO level measurement in adult SCC patients in the acute setting of the emergency department [11]. The results suggested that low initial NO levels were associated with higher pain scores, while higher initial NO levels were associated with lower pain scores. This suggested a relationship between NO metabolite levels and the patient's clinical presentation in SCC. The present study was undertaken to determine if 1) the initial NO measurement could predict pain improvement in the ED, and 2) serial NO measurements could serve as a marker for pain improvement during the course of ED analgesic treatment.

We found that patients with little or no change in the pain score during the course of ED treatment had significantly low initial NO levels. The low initial NO level seen in the persistent pain group may reflect a subset of patients who are unable to adequately vasodilate in response to their tissue ischemia, leading to a higher degree of tissue ischemia and, therefore, more pain. Conversely, higher initial NO levels were seen in patients experiencing pain relief. In the clinical setting, ischemia and tissue hypoxia is generally manifested as pain [25]. An elevated plasma nitrite/nitrate level suggests an increase in endogenous NO production [26], perhaps indicating a more vasodilated state. The diminished pain score in the group with the high NO metabolite level may reflect a compensatory, more vasodilated state in response to vascular occlusion and tissue ischemia. The enhanced vasodila-

tion leads to improved blood flow and increased oxygenation at the tissue level, thereby decreasing painful ischemia. In the face of ischemia, increased NO with a resultant vasodilation is a well-described response to myocardial [27], neurological [28], and gastrointestinal [29] ischemia. Pain and NO have been linked, but it is unclear whether NO in and of itself can cause or relieve pain [30]. The few studies available examining the role of NO and pain appear to support NO as either evoking [31] or attenuating [30] pain. Further research is needed to clarify the role of NO in pain.

The measurement of NO metabolites in patients in SCC may have potential as an objective method of measuring SCC pain and possibly predicting the clinical course in the ED. Rheological studies looking at erythrocyte deformability [21], blood yield stress, and erythrocyte endothelial adhesion have been used to assess and monitor SCA patients [32]. In addition, measurements of acute inflammatory phase proteins such as C-reactive protein and serum amyloid A protein have been investigated [33]. However, these tests are neither sensitive or specific nor readily available or practical in the acute setting. Interestingly, we found, in a subset of patients with multiple emergency department visits, a significant negative relationship between initial NO level and initial pain score. This may suggest, for certain patients, that NO levels could be used as a marker for pain.

We were unable to find a significant correlation between absolute NO level and absolute pain score, nor were we able to establish a connection between changes in NO level and change in pain levels during the ED treatment of our subjects. The four to six hour study time frame for our patients may be too short a time to see any measurable changes. Indeed, the trend towards higher NO levels with increased duration of SCC pain prior to the ED visit lends some support to the need for a longer time period. In addition, prior studies have suggested that NO levels appear to increase over a four to seven day period [34]. Future studies should examine serial measurements of NO levels both in the ED and either during the course of hospitalization or as out-patient follow-up.

Our study has several limitations. First, patients were not standardized in terms of duration of pain prior to entry, location of pain, and prior analgesic therapy. These factors could certainly influence the actual NO metabolite measurement as well as the interpretation of the particular level. Second, the subjective nature of pain has inherent difficulties regarding its evaluation. Third, because of the difficulty and impracticality of measuring actual tissue ischemia, we were unable to quantify the actual amount of tissue ischemia present in each patient. Finally, the diets of the patients enrolled were uncontrolled. Diets high in vegetables have been shown to alter nitrite and nitrate levels in healthy volunteers [35].

CONCLUSION

Initial elevated NO metabolite levels are associated with lower pain scores in SS patients presenting to the ED with acute SCC. Patients with lower levels are associated with higher pain scores, suggesting less compensatory vasodilation. NO metabolites may potentially represent a marker for compensatory mechanisms in SCC tissue ischemia. Further study is needed to delineate the exact mechanisms involving NO and SCC.

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